

# Polymorphism Detection of some Transcription Factors Genes Conferring Drought Tolerance in Diverse Wheat (*Triticum aestivum* L.) Genotypes

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## ABSTRACT

Drought is considered the most hazardous environmental abiotic stress factors, which adversely affects wheat production (*Triticum aestivum* L.). Several transcription factor (TF) families, such as WRKY, MYB, and NAC, have an important role in response to drought stress in plants. The investigation of NaC and WRKY genes is considered a new area for research, especially in Egypt. Thus, this study aimed to detect the three genes namely, TaNAC2a, TaWRKY2, and TaWRKY19 by using DNA marker based on encoding regions in different wheat genotypes. In addition to that, to identify the polymorphism in TaNAC2a gene by performing sequence analysis of the central part of exon 2 in seven wheat cultivars. The TaNAC2a, TaWRKY19, and TaWRKY2 genes were detected in most of the tested wheat genotypes. The polymorphic bands detected for TaNAC2a, TaWRKY19, and TaWRKY2 suggest the existence of other alleles at the same locus of these genes. The central part of exon2 was sequenced (260 bp) showing the conserved sequence in all cultivars, except the positions from 813 to 863 were high polymorphic. Detected SNPs related to drought tolerance were not found. The amino acids sequence encoded from the target DNA region was highly conserved from position 282 to 319 in the N-terminal domain in all genotypes except a few positions. Detection of these important genes is considered a critical start point for further study especially gene expression of these genes.

**Keywords:** Drought tolerance, NAC genes, Polymorphism, WRKY genes, Wheat.

## INTRODUCTION

Wheat is a staple food for more than one-third of the world's population and crops yields are significantly reduced by global climatic changes, Drought is the most important of these changes (Anjum *et al.*, 2011 and Hasanuzzaman *et al.*, 2018).

Drought is considered the most hazardous environmental abiotic stress factors, which adversely affects wheat production (*Triticum aestivum* L.) (Abebe *et al.*, 2010 and Bi *et al.*, 2016). It has caused an average of 13.7% loss in cereal production worldwide

over the past few decades (Lesk *et al.*, 2016). Several transcription factors (TF) families, such as WRKY (WRKYGQK), MYB (myeloblastosis-related proteins), and NAC (NAM, ATAF, and CUC), have an important role in response to drought stress in plants (Li *et al.*, 2020).

WRKY TFs are involved in signal transduction pathways by regulating the transcription of target genes leading to stress tolerance in plants (Chen & Zhu, 2004; Yamaski *et al.*, 2005; Budak *et al.*, 2013; Tripathi *et al.*, 2014 and Wang *et al.*, 2018). WRKY domain characterizes the WRKY family, it consists of a short-conserved sequence WRKYGQK at the N-terminal and a zinc finger motif at the C-terminal (Rushton *et al.*, 1995 and Eulgem *et al.*, 2000). The WRKY family is divided into three groups based on the number of WRKY domains and zinc-finger structures (Yousfi *et al.*, 2016). The transcription of the target genes is regulated by the WRKY domain through binding to the upstream W-box sequence of these genes (Zhang *et al.*, 2016 and Wu *et al.*, 2017). It was found that wheat contains 171 WRKY TFs, some of them tested for their function (Ning *et al.*, 2017). Recently, it was reported that TaWRKY TFs are involved in various biotic and abiotic stress responses, such as pathogen defense, high salt stress, extreme temperature, drought stress, and senescence (Qin *et al.*, 2015; Wang *et al.*, 2015; Kage *et al.*, 2017; Wang *et al.*, 2017 and Li *et al.*, 2020).

In transgenic Arabidopsis plants, TaWRKY2 and TaWRKY19 were overexpressed increasing drought tolerance. It was also found that TaWRKY2 had induced STZ and RD29B gene expressions. In addition, transgenic plants containing TaWRKY19 had higher expression levels of DREB2A, RD29B, Cor6.6, and RD29A genes (Niu *et al.*, 2012). Many TaWRKY genes such as TaWRKY1 and TaWRKY33 were transferred to Arabidopsis revealing drought and heat resistance (He *et al.*, 2016).

Multiple Regulatory cis-elements are identified in the TaWRKY2 promoter, it was activated by drought. TaWRKY2-overexpressing transgenic wheat lines

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showed an increase in drought tolerance and grain yield in wheat, thus providing a promising candidate target for improving the drought tolerance of wheat cultivars through genetic engineering (Gao *et al.*, 2018).

NAC superfamily is a TF family distinguished by a conserved domain at the N-terminus and a variable domain regulating transcription at the C-terminus and revealed an important role in response to biotic and abiotic stress (Tang *et al.*, 2012 and Nakashima *et al.*, 2014).

It was noticed that NAC genes regulated by abiotic stress such as AtNAC2, ZmSNAC1, and OsNAC78 in Arabidopsis, maize, and rice respectively (He *et al.*, 2005; Moumeni *et al.*, 2011 and Lu *et al.*, 2012). Moreover, overexpression of NAC genes such as OsNAC5, OsNAC9, and OsNAC10 induced drought tolerance in rice (Jeong *et al.*, 2010 & 2013 and Redillas *et al.*, 2012), GmNAC11 and GmNAC20 induced salt tolerance in soybean (Hao *et al.*, 2011).

In wheat overexpression of NAC genes increased drought tolerance in transgenic plants of tobacco. Overexpression of TaNAC2 and TaNAC67 in transgenic Arabidopsis enhanced tolerances to abiotic stresses (Mao *et al.*, 2012 and 2014). TaNAC8 is induced by both abiotic and biotic stresses (Xia *et al.*, 2010a and 2010b). Transgenic wheat lines and Arabidopsis containing TaSNAC8-6A exhibited drought tolerance (Mao *et al.*, 2020).

DNA markers based on non-coding sequences such as RAPD (Random Amplified Polymorphic DNA) (El-Sayed & Ibrahim, 2008 and El-Sayed & Rafudeen, 2012), SSR (Simple Sequence Repeats) (Sadat *et al.*, 2013 and Kim *et al.*, 2014) and ISSR (Inter Simple Sequence Repeats) (El-Sayed and Ibrahim, 2008) were used to study genes which are responsible of drought tolerance. Recently, encoding gene sequences associated with transcriptional factors have been used (Rana *et al.*, 2013). Thus, DNA markers either based on coding or non-coding sequences are applied in marker-assisted selection (Kosova *et al.*, 2014; Lakhneko *et al.*, 2016; Pokhylo *et al.*, 2016 and Stepanenko *et al.*, 2017).

The investigation of NAC and WRKY genes is considered a new area for research, especially in Egypt; few research related to these genes is available in Egypt. This study aims to detect TaNAC2a, TaWRKY2, and TaWRKY19 in different wheat genotypes, and identify the polymorphism in TaNAC2a gene among some Egyptian wheat genotypes.

## MATERIAL AND METHODS

### Plant material:

Twenty-nine wheat genotypes (*Triticum aestivum*) were used in this study representing different levels of drought tolerance listed in Table (1).

They consisted of nineteen Egyptian wheat genotypes, two genotypes Obtained from CIMMYT (International Maize and Wheat Improvement Center) ; Veery, and Pavon 76, and five wheat somaclonal variant lines (Soma 1, Soma 2, Soma 8, Soma 10, and Soma16) which were previously developed by in vitro selection for drought tolerance, using different concentrations of abscisic acid (ABA) obtained by Wahba (2010), as part of a previous research project (BIOT 6), conducted in the Biotechnology Laboratory, Crop Sciences Department, Faculty of Agriculture, Elshatby, Alexandria University, Egypt, Moreover, two internationally accredited heat stress tolerant wheat genotypes (Ksu 105 and Ksu 106) (Barakat *et al.*, 2011) and the Sudanese wheat cultivar Debaira is used as a reference for genotypes evaluating towards drought tolerance.

### DNA isolation:

For each genotype, the seeds were selected and germinated for two weeks. Then genomic DNA was extracted from leaves by the CTAB method (Saghai – Maroof *et al.*, 1984).

DNA concentration of wheat genotypes was determined using (Ultraspec. 1000®, UV/spectrophotometer, Pharmacia Biotech®) the concentration of DNA was calculated, according to Sambrook *et al.* (1989).

### PCR conditions and sequencing:

The TaNAC2a, TaWRKY2, and TaWRKY19 genes were detected by amplification of encoding region in these genes by using specific primers (as previously described by Lakhenko *et al.* (2018)) listed in Table (2). All genotypes were exposed to these primers.

PCR reaction was performed in a total volume of 25 µL, including 50 ng genomic DNA, 10 uL of 2 X TOP™ simple DyeMIX-nTaq PCR, 1.5 µL, 0.5 µM of each primer. It was carried out using the following programs: initial denaturation at 94 °C for 5 min.; 35 cycles of 94 °C for 30sec., annealing at 58 °C- 61 °C for 30sec., 72 °C for 30 sec., and a final extension at 72 °C for 5 min. The amplified products were electrophoresed on 2% agarose gels.

20 µL of PCR products resulting from amplification by primer pair of TaNAC2a gene for seven wheat cultivars: (Shandweel-1, Sakha95, Sakha94, Misr3, Giza171, Gemmiza9, and Gemmiza12), were sent to MacroGen Company, (API3730XL DNA Analyzer, Applied Biosystems) for sequencing analysis.

**Table 1. Pedigree of wheat genotypes used in this study**

S.n.	Genotypes	Pedigree	Origin
1	Giza 168	MIL/BUC//SERI	Egypt
2	Giza 171	SAKHA 93 / GEMMEIZA 9	Egypt
3	Sakha 92	NAPO63/TNT1A66//WERN “S”	Egypt
4	Sakha 93	SAKHA92/TR810328	Egypt
5	Sakha 94	OPATA/RAYON//KAUZ	Egypt
6	Sakha 95	PASTOR//SITE/MO/3/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/4/WBLL1	Egypt
7	Gemmeiza 9	ALD “S”/HUAC“S”//CMH74A.630/SX	Egypt
8	Gemmiza 11	B0W“S”/KVZ“S”//7C/SERI82/3/GIZA168/SAKHA61.	Egypt
9	Gemmiza12	OTUS/3/SARA/THB//VEE.	Egypt
10	Misr 1	OASIS/SKAUZ//4*BCN/3/2*PASTOR	Egypt
11	Misr 2	SKAUZ/BAV92	Egypt
12	Misr 3	ATTILA*2/PBW65*2/KACHU	Egypt
13	Sids 6	MAYA“S”/MON“S”//CMH74A.592/3/SAKHA8*2SD10002-4SD-3SD-1SD-0SD	Egypt
14	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160147/3/BB/GLL/4/CHAT “S”/6/MAYA/VUL	Egypt
15	Sids 13	KAUZ “S” //TSI/SNB “S”	Egypt
16	Sids 14	SW8488*2/ KUKUNA	Egypt
17	Shandaweel- 1	SITE//MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC.	Egypt
18	Nubaria 1	OASIS / 5 /*BOR95/5 / CNDO/R134//ENTE/MEX175/3/ CNDO/ R143	Egypt
19	Debiera	HD-2160/5/TOBARI-66/CIANO-67//BLUEBIRD/3/NAINARI-60*2//TOM-THUMB/SONORA-64/4/HD-1954; HD-2160/5/TOBARI-66/CIANO-67//BLUEBIRD/4/NAINARI-60*2//TOM-THUMB(TT)/SONORA-64/3/LERMA-ROJO-64/SONORA-64. HD-2172; HYBRID-DELHI-2172; ESWYT-40; DEBIERA	Sudan
20	Sahel 1	N.S.732/PIMA//VEE“S”.CR735	Egypt
21	Veery	Kavkaz/Buho//Kalyansona/Bluebird	CIMMYT
22	Pavon 76	Vicam-71//Ciano-67/Siete-Cerros-66/3/Kalyansona/Bluebird	CIMMYT
23	Ksu105	King Saud University	Saudia Arabia
24	Ksu 106	King Saud University	Saudia Arabia
25	Soma1	A somaclonal variant derived from Sakha 69	Egypt
26	Soma2	A somaclonal variant derived from Sakha 69	Egypt
27	Soma 8	A somaclonal variant derived from Sakha 69	Egypt
28	Soma 10	A somaclonal variant derived from Sakha 69	Egypt
29	Soma 16	A somaclonal variant derived from Sakha 69	Egypt

**Table 2. Primers pairs sequences and annealing temperatures**

Gene name	Forward Primer sequence (5'–3')	Reverse Primer sequence (5'–3')	Annealing temperature
TaNAC2a	F: GGTAGTGCGGTGCTTCCAAT	R: TGAATGTTGTTGCTCGTCCC	58°C
TaWRKY2	F: GGCGTGCCGACGTCATCTT	R: AGCAGAGGAGCGACTCGACGA	61°C
TaWRKY19	F: AGGGAAGCATACGCATGACGTGC	R: GGCGAGATCGTTCAGAATGGCTGT	60°C

**Bioinformatic analysis:**

NCBI (National Center for Biotechnology Information), UniProt (Universal Protein resource), and Ensembl plants Databases were searched for information about TaNAC2a, WRKY2, and WRKY 19. NCBI searched for accessions of DNA, mRNA, and Protein (<https://www.ncbi.nlm.nih.gov/>). Ensembl plants searched for the detection of Exons and Introns (<http://plants.ensembl.org/index.html>). UniProt searched for protein domain determination (<https://www.uniprot.org/>).

Samples of DNA sequences of the central part of exon 2 of the TaNAC2a gene were aligned with the reference gene sequence with GenBank accession number of HM027575.2 by using nucleotide blast at NCBI database (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

BLASTX (DNA vs protein) search was used to identify amino acids sequence which aligned with the reference sequence of Gen bank accession number ADE34618 348 aa. Amino acid translations of the nucleotide sequences of transcripts are determined using the ExpASY portal (<http://web.expasy.org/translate>).

Multiple sequence alignments of the sequenced DNA samples and predicted amino acid sequences from the seven wheat cultivars were conducted using the tool provided by the European Bioinformatics Institute (<https://www.ebi.ac.uk/Tools/msa/clustalo/>).

Additionally, the MEGA11 program (<https://www.megasoftware.net/>) was utilized to construct a phylogenetic tree based on amino acid sequences by using the Neighbor-joining method to analyze the genetic variation in this gene among the seven wheat cultivars.

**RESULTS AND DISCUSSION**

WRKY and NAC transcription factors are involved in the signal transduction pathway by regulating the transcription of drought-tolerant genes which leads to drought tolerance. Consequently, drought tolerance and high grain yield can be enhanced by TaWRKY19, TaWRKY2, and TaNAC2a, these genes represent a promising target to improve drought tolerance in wheat (Mao *et al.*, 2012; Niu *et al.*, 2012 and Gao *et al.*, 2018). Therefore, this study was performed to detect these

genes in diverse wheat genotypes. DNA markers based on encoding regions of these genes are used to detect these genes.

**Characterization of TaNAC2a, TaWRKY19 and TaWRKY2 genes :**

Valuable information about the tested genes and the amplified encoding region was obtained by searching NCBI, Ensemble plant, and UniProt databases and by Kumar *et al.* (2018). The gene of **TaNAC2a** comprises 2 exons and 1 intron and allocates at the short arm of the 7A chromosome. According to analysis, the two exons that compose the gene in concern are responsible for producing a 348-amino-acid protein, which is the result of the gene's expression. The expected amplified region of this gene, generated by the polymerase chain reaction using the specific primer pair, annealed at the central part of exon 2, and gave 227 bp fragments. The Start position is 753 and the end position is 979. The related encoded sequence length is 75 amino acids, the start position is 249 and the end position is 323.

**TaWRKY19** gene comprises 4 exons and 3 introns and allocates at the short arm of 2B chromosome, as clearly explained. According to data, the four exons that make up the gene in concern are responsible for the gene's production of a 468-amino-acid protein. The used primer pair for this gene annealed at exon 4 and gave 160 bp fragments. The Start position for this region is 1241 bp and the End position is 1400. The sequence under consideration plays a role in the creation of the 53-amino-acid protein. The start position is 415 and the end position is 467.

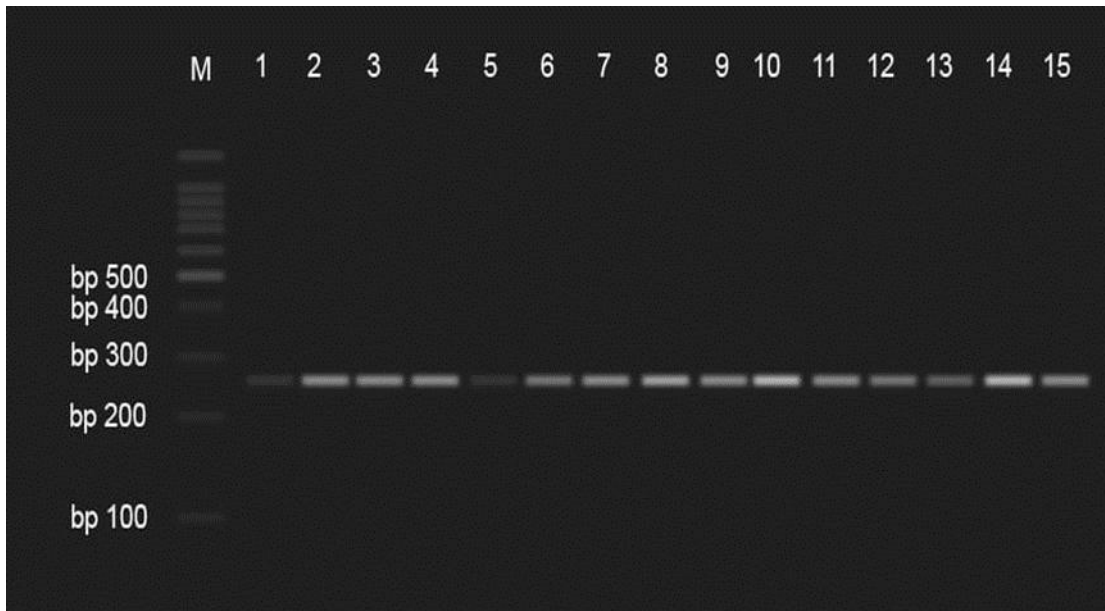
As previously proven, **TaWRKY2** comprises 4 exons and 3 introns and allocates at the short arm of the 1D chromosome. According to research, the four exons that make up the gene in question are responsible for creating a 468-amino-acid protein that is produced by the gene itself. The DNA sequence of interest is in the fourth exon of the gene under inquiry. The used Primer pair for this gene locus annealed at exon 4 and gave 188 bp fragments. The start position is 1217 and the end position is 1404. According to bioinformatics analyses the sequences under examination are involved in the synthesis of the 62-amino-acid protein. The start position is 407 and the end position is 468.

Therefore, the importance of these sequences is that they are part of the structure of the protein that is produced as a result of the gene expression of the gene under study. Because these sequences are important and indicate the presence of the gene in the genotype under study, the researchers utilized these primers to detect them.

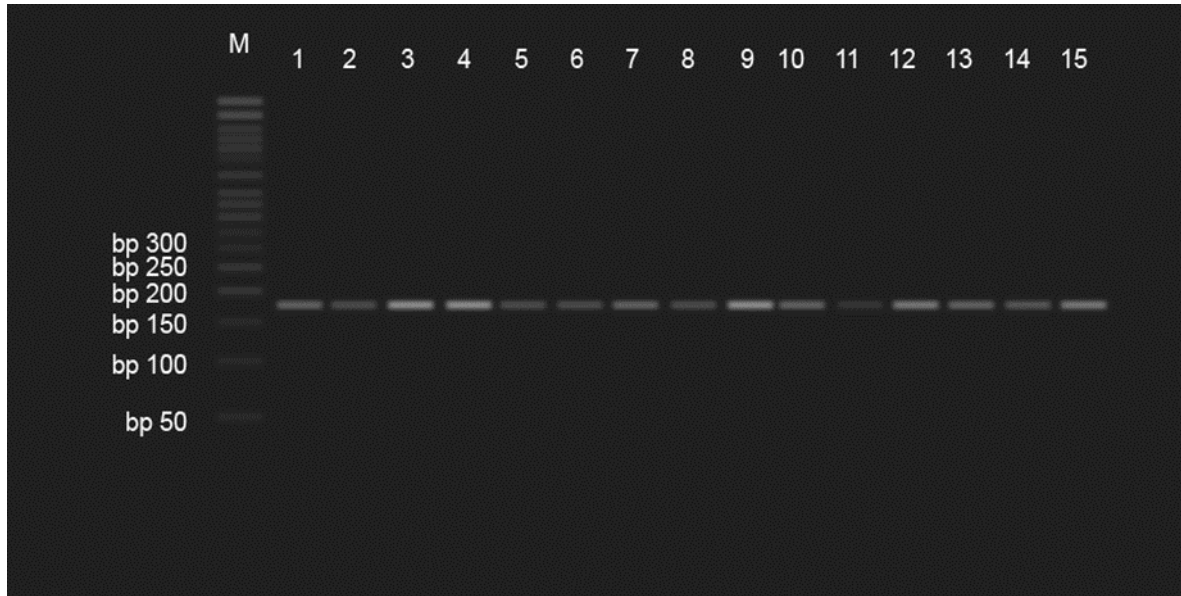
## 2. Detection of TaNAC2a, TaWRKY2 and TaWRKY19 genes in thirty wheat genotypes

DNA markers based on encoding sequences representing unique regions are used to detect polymorphism in this region for TaNAC2a, TaWRKY2, and TaWRKY19 genes in thirty wheat genotypes. TaNAC2a region amplified by using a specific primer pair, the electrophoretic pattern showed PCR-products of DNA marker as fragments approximately 260 bp (Figure 1) in length. TaNAC2a locus detected in all

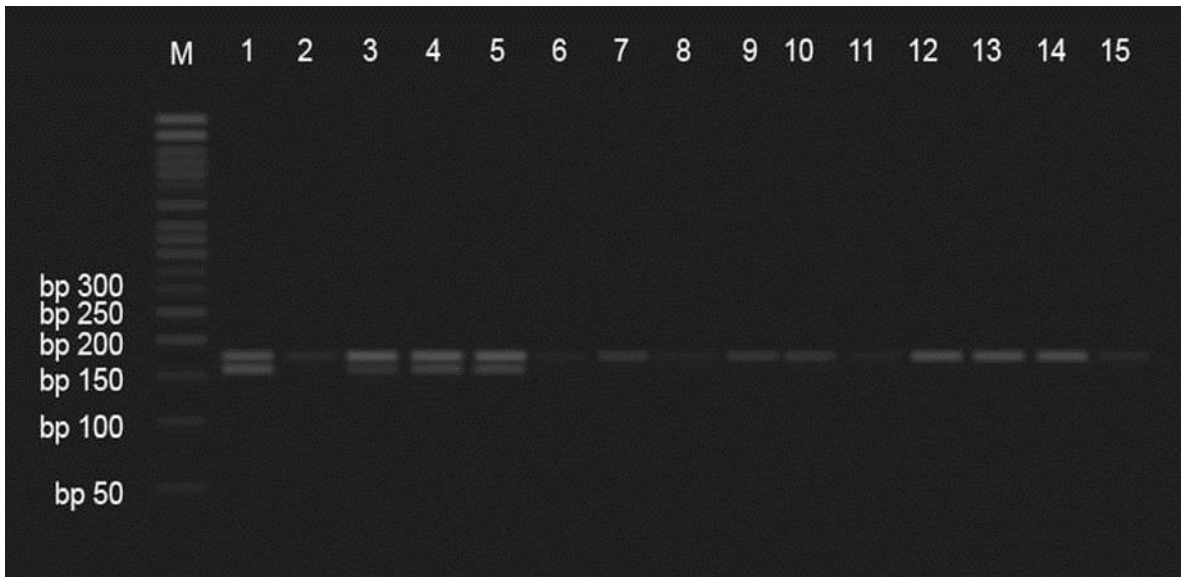
genotypes except in Gemmeiza 11, Giza171, Sakha92, Sakha93 and Nubaria1. The amplified locus of TaWRKY19 gave DNA marker fragment approximately 175 bp in all genotypes (Figure 2) except in, Pavon, and Soma 8. TaWRKY 2 region amplified using specific primer pair in all genotypes. the electrophoretic pattern showed PCR-products segregation of DNA marker as fragment approximately 175 bp in length in the most genotypes, while Gemmeiza11, Misr3, Nubaria1, Debeira, Pavon, Ksu105, Ksu106, Soma2 and Soma8 did not reveal any fragments, some genotypes revealed two fragments approximately 160 bp and 175bp (Figure3): Misr1, Misr2, Giza168, Sids14, Soma1 and Soma16. It deserves to be mentioned that TaNAC2 and Ta WRKY2 genes not detected in Gemmeiza 11 and Nubaria1. Ta WRKY19 and Ta WRKY2 genes not detected in Pavon, and Soma8.



**Figure 1.** The electrophoretic pattern showing 260bp polymorphic band as DNA marker of TaNAC2a. Lanes from 1 to 15 representing the tested wheat genotypes: Gemmiza9, Gemmiza12, Sids13, Sids14, Giza168, Debeira, Sahel-1, Shandweel-1, Veery, Ksu105, Ksu106, Soma2, Soma8, Soma,10, Soma16



**Figure 2.** The electrophoretic pattern showing 175bp polymorphic band as DNA marker of TaWRKY19. Lanes from 1 to 15 representing the tested wheat genotypes: Gemmiza9, Gemmiza12, Sids13, Sids14, Giza168, Debeira, Sahel1, Shandweel-1, Sakha 95, Veery, Ksu105, Ksu106, Soma2, Soma,10, Soma16



**Figure 3.** The electrophoretic pattern showing 150 and 175bp polymorphic bands as DNA marker of TaWRKY2. Lanes 2 and from 6 to 15 representing the tested wheat genotypes with 175bp polymorphic bands: Gemmiza9, Gemmiza12, Sids13, Sids14, Giza168, , Sahel1, Shandweel-1, Sakha95, Veery, , Soma2, and Soma10. Lanes 1 and from 3 to 5 representing the tested wheat genotypes with 150 and 175bp polymorphic bands: Misr1, Misr2, Giza168 and Soma16

This study showed that the TaNAC2a gene was detected by the presence of a polymorphic band representing approximately 260 bp fragments in most genotypes except Gemmeiza 11, Giza171, Sakha92, Sakha93 and Nubarial1. This result was in agreement with the results obtained by Abd El-Moneim *et al.* (2020) who found that TaNAC genes were

characterized well in Shandaweel- 1, Sakha 95, and Misr 2, which were included in our study, and these genes may be used as markers to select tolerant genotypes. Likewise, TaWRKY 19 was detected by the presence of a polymorphic band representing approximately 175 bp fragment in most genotypes, this band was absent in Pavon, and Soma 8, indicating that



these genotypes do not have TaWRKY 19. In contrast, Lakhneko *et al.* (2018) found that no polymorphism was detected for TaNAC2a and TaWRKY19. In addition, TaWRKY2 was detected by polymorphic band representing approximately 175 bp fragment of DNA marker in most genotypes, some genotypes including Misr1, Misr2, Giza168, Sids14, Soma1 and Soma16 revealed two fragments approximately 160 bp and 175bp. On the other hand, Gemmeiza11, Misr3, Nubaria1, Debeira, Pavon, Ksu105, Ksu106, Soma2 and Soma8 did not reveal any fragments. Abd El-Moneim (2019) reported that TaWRKY2 revealed high expression under drought stress in Shandweel-1 showing similar results concerning Shandweel-1. Similarly, Lakhneko *et al.* (2018) detected two polymorphic bands for TaWRKY2 in a set of wheat and rye varieties, old landraces, and interspecific hybrids. Forty-three TaWRKYs were identified by Niu *et al.* (2012) through analysis of wheat ESTs. Thus, the polymorphic bands detected for TaNAC2a, TaWRKY19, and TaWRKY2 genes suggest the existence of other alleles at the same locus of the gene as shown by Lakhneko *et al.* (2018). Although, Pavon76 and Soma 8 do not possess TaNAC2a and TaWRKY2a genes, they are tolerant and intermediate tolerant (Hamza *et al.*, 2018) respectively. This result is attributed to the complicated nature of the inheritance of drought tolerance and the presence of other genes involved in drought tolerance. The impact of drought stress differs based on the genotype, environment, and genotype-environment interaction (Hoffman *et al.*, 2009). In addition, drought tolerance is a complicated trait inherited quantitatively, and controlled by multiple agronomic traits and polygenes (Mwadingeni *et al.*, 2017). On the other hand, some intolerant genotypes such as Gemmeiza 9 and Gemmeiza 12 (Hamza *et al.*, 2018) containing TaWRKY19, TaWRKY2, and TaNAC2a, this result may be attributed to the blocking of gene expression in these genotypes as a result of genetic background variation and complicated nature of the trait as mentioned above.

The primer pair of TaWRKY2 gene locus gave 188 bp band and annealed at exon 4 for GenBank accession number EU665425, whereas the obtained band in this study gave 160 and 175 bp. The primer pair of TaWRKY19 gene locus gives 160 bp band and annealed at exon 4 for GenBank accession number EU665430, while the polymorphic band obtained in this study gave 175bp. The primer pair of TaNAC2a gene locus gives a

227 bp band and annealed at the central part of exon2 for GenBank accession number HM027575.2, while the obtained polymorphic band gave 260 bp in the current study. Thus, the length of the obtained amplified regions for all genes in this study differed from the expected amplified regions of these genes in the gene bank, this result may be attributed to Indel mutations. These obtained results are critical for further investigations of wheat drought tolerance. Screening for these genes was the first step to ensure that the tested genotypes contained them to facilitate further research.

### 3. Characterization of the central part of exon2 of TaNAC2a gene:

NAC is considered one of the largest TFs families in plants (Ernst *et al.*, 2004; Gong *et al.*, 2004 and Xiong *et al.*, 2005). The family members have a highly conserved N-terminal NAC domain and a variable C-terminal transcriptional regulation domain, polymorphic in both length and sequence. Therefore, TaNAC2 has the potential for utilization in transgenic breeding to improve abiotic stress tolerances in crops (Ernst *et al.*, 2004; Olsen *et al.*, 2005 and Mao *et al.*, 2012). This transcription factor plays an important role in stress responses and developmental processes. Six genes (named TaNAC2a, TaNAC4a, TaNAC6, TaNAC7, TaNAC13, and TaNTL5) encoding NAC TFs were discovered in wheat (*Triticum aestivum*) which possessed a potential role in improving stress tolerance and the regulation of development in wheat (Tang *et al.*, 2012).

TaNAC2a gene plays an important role in the regulation of gene expression of the genes involved in drought tolerance. Consequently, this study aimed to analyze the sequence of the central part of exon2 of the TaNAC2a gene to detect the polymorphism in this region in seven Egyptian wheat cultivars.

Based on the obtained sequencing data, the 260bp fragment of the central part of exon2 of the TaNAC2a gene in each cultivar was aligned with the reference sequence of GenBank accession number HM027575.2. Sequence alignment of each wheat cultivar with reference sequence showed high identity more than 90% as follows: 94.2%, 94.2%, 95%, 96%, 97.7%, 97.7% and 97.1% in Gemmeiza 9, Sakha 94, Giza171, Sakha95, Misr3, Gemmeiza12, and Shandweel-1 respectively, Samples sequences registered with Gene bank accession numbers as shown in Table (3).

**Table 3. Gene bank accession numbers and the identity of sequence of interest of wheat cultivars aligned with reference sequence**

S.n.	Cultivar	Gene bank accession numbers	Identity
1.	Gemmeiza9	PP471471	94.21%
2.	Sakha94	PP471472	94.24%
3.	Giza171	PP471473	95%
4.	Sakha95	PP471474	96.15%
5.	Misr3	PP471475	97.74%
6.	Gemmeiza 12	PP471476	97.74%
7.	Shandweel-1	PP471477	97.14%

Multiple sequence alignment of the genotypes and the reference gene was conducted, and Analysis of single nucleotide polymorphisms (SNPs) was performed. 260 bp sequenced region showing the conserved sequence in all cultivars, except from position 813 to 863 were high polymorphic. SNPs were detected: there is one SNP at position g.804 C/-distinguishes the Shandweel-1 cultivar where C allele in reference gene deleted in Shandweel-1. Gemeiza 9 was different from other genotypes in the following positions: g. 827C > G, 833C > A and 837 A > G where C allele mutated to G allele, C mutated to A, and A mutated to G respectively. Giza 171 was different from other genotypes in 893 G > A, 972 C > A, and 973 A > C where G mutated to A, C mutated to A and A mutated to C respectively this result needs to confirm by using more genotypes. On the other hand, SNPs related to drought tolerance were not detected. Sequencing of full length of the gene may reveal SNPs correlated with drought tolerance.

Amino acids sequence paralleled to DNA sequence predicted and aligned to protein with Gene bank Accession number ADE34618 348 aa using blastx, then multiple alignment of translated region performed using clustal omega. Amino acid sequences encoded from the target DNA region were highly conserved from position 282 to 319 in the N-terminal domain in all genotypes except in a few positions. This result agreed with the results obtained by Ooka *et al.* (2003) and Mao *et al.* (2012) who found that the N-terminal region containing NAC domain (DNA binding domain) was highly conserved across monocots and dicots.

Unique amino acids were detected in Gemmeiza- 9: Alanine, Serine, Glycine, Histidine, and Threonine at the positions 280,281,283,294and 308 respectively, while Tyrosine, Asparagine, Glutamine, Glutamine, and Serine located at the same positions respectively in the other genotypes. In addition, unique amino acids detected in Giza 171: Aspartic acid, Lysine, Glutamine, and Histidine at the positions 295,321,322 and 323 respectively while Glutamine, Asparagine, Asparagine, and Isoleucine located at the same positions respectively in the other genotypes (Figure 4). Furthermore, Cysteine and Leucine were unique amino acids for Gemmiza-9 and Sakha 94 at position 278 and 289 respectively, while Serine and Histidine were found at the same positions respectively in the other genotypes. Arginine distinguished Giza 171 and Sakha 95 at position 320, while Serine was found at the same position in the other genotypes.

The phylogenetic tree constructed based on amino acid sequences is divided into two main subgroups, one of them including Sakha94 and Gemmeiza 9 showing high similarity between both of them in this region compared to other genotypes and another group including Misr3, Shandweel-1, Gemeiza-12, Giza171, and Sakha 95, high similarity was found among Misr3, Gemeiza-12, and Shandweel-1 compared to Giza171, and Sakha 95 (Figure 5). It was noticed that the phylogenetic tree is not segregated into groups according to drought tolerance levels.



Reference	SVYNNQ	QQQ	H	SAEQGF	VGVDPGSGT	PS	60
Misr3	SFYNNQ	QQQ	H	SAEQGF	VGVDPGSGT	SS	43
Gemeiza-12	SFYNNQ	QQQ	H	SAEQGF	VGVDPGSGT	SS	43
Shandweel-1	SFYNNQ	QQQ	H	SAEQGF	VGVDPGSGT	SS	42
Giza-171	SFYNNQ	QQQ	H	SAEQDF	VGVDPGSGT	SS	43
Sakha-95	SFYNNQ	QQQ	H	SAEQGF	VGVDPGSGT	SS	43
Gemeiza-9	CFASNG	QQQ	L	SAEHGF	VGVDPGSGT	ST	45
Sakha-94	CFYNNQ	QQQ	L	SAEQGF	VGVDPGSGT	SS	45

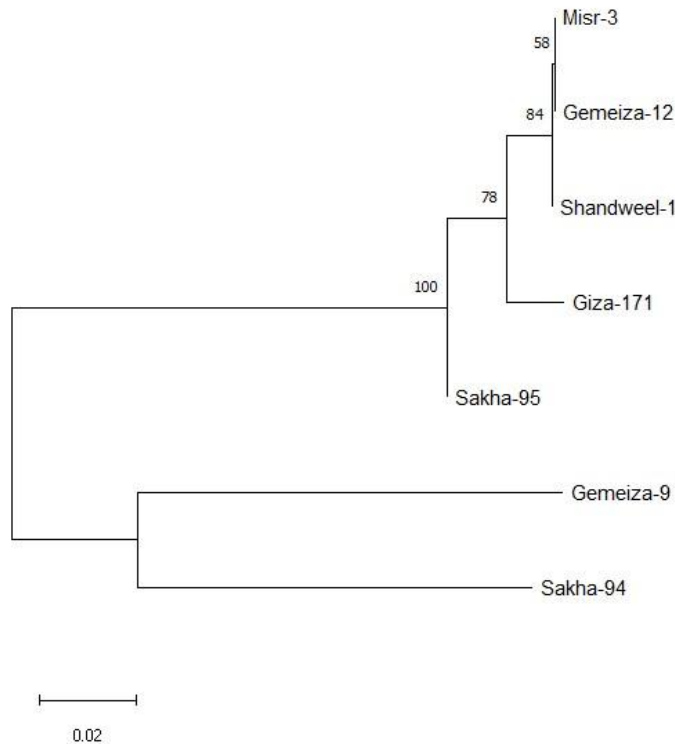
Reference	IVSDEDVLTGT	SNNI	75
Misr3	IVSHEDVLTGT	SNNI	60
Gemeiza-12	IVSHEDVLTGT	SNNI	60
Shandweel-1	IVSHEDVLTGT	SNNI	58
Giza-171	IVSHEDVLTGT	RKQH	59
Sakha-95	IVSHEDVLTGT	RNNI	59
Gemeiza-9	IVSHEDVLTGT	SNNI	64
Sakha-94	IVSHEDVLTGT	SNNI	64

**Figure 4. Multiple alignment of amino acids sequence which encoded from central part of exon 2of TaNaC2a gene using Clustal Omega for seven wheat cultivars.**

**Reference:** Reference sequence of Gene bank Accession number ADE34618 348 aa.

**Red letters:** Unique amino acids at the following positions from left to right direction respectively:278,280,281,283,289,294,295,308,320,321,322, and 323

**Yellow background region:** conserved region



**Figure 5. Phylogenetic relationship of the central part of TaNaC2a among Wheat genotypes: the phylogenetic tree constructed by using Neighbor-joining method**

## CONCLUSION

The three genes; TaNAC2a, TaWRKY19, and TaWRKY2 were detected in most of the tested wheat genotypes, and the obtained result revealed polymorphism in all genes loci. The polymorphic bands detected for TaNAC2a, TaWRKY19, and TaWRKY2 genes suggest the existence of other alleles at the same locus of the gene. Detection of these important genes is considered a critical starting point for further study, especially gene expression of these genes. 260 bp sequenced region, containing the central part of exon2, showing the conserved sequence in all cultivars, except the positions from 813 to 863 were high polymorphic. SNPs were detected, while SNPS related to drought tolerance were not detected. Amino acids sequence encoded from the target DNA region was highly conserved from position 282 to 319 in the N-terminal domain in all genotypes except a few positions. The phylogenetic tree constructed based on amino acid sequence showed that there is a high similarity between Sakha 94 and Gemmeiza 9, and it did not reveal segregation based on drought tolerance. The three genes; TaNAC2a, TaWRKY19 and TaWRKY2 detected in this study might be further considered in wheat breeding programs and might be useful for developing improved cultivars for the improvement of drought tolerance.

## Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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## الملخص العربي

### اكتشاف تعدد أشكال تواجد بعض جينات عوامل النسخ التي لها دور هام في تحمل الجفاف في بعض التراكيب الوراثية المتنوعة لنبات القمح (*Triticum aestivum* L.)

هدى شكم ، محمد عبيد ، دعاء حمزة

في معظم الطرز الوراثية للقمح التي تم اختبارها، كما أن ال TaNAC2a polymorphism المكتشف لجينات TaWRKY19 و TaWRKY2 تشير إلى وجود أليات أخرى لهذه الجينات. يعتبر الكشف عن هذه الجينات المهمة نقطة بداية هامة لمزيد من الدراسة وخاصة التعبير الجيني لهذه الجينات. وظهرت النتائج ان قطعة ال DNA التي يبلغ طولها ٢٦٠ bp المشتملة على المنطقة المركزية من exon2، تتابعها محفوظ في جميع الأصناف، باستثناء الموقع ٨١٣ إلى ٨٦٣ فكانت متغيرة بدرجة عالية. لم يتم العثور على SNPs متعلقة بتحمل الجفاف. ووجد أن تسلسل الأحماض الأمينية المشفر من منطقة الحمض النووي المستهدفة كان محفوظ بشكل كبير من الموضع ٢٨٢ إلى ٣١٩ في الطرف N-terminal في جميع الأنماط الجينية باستثناء عدد قليل من المواقع.

يعتبر الجفاف من أخطر عوامل الإجهاد البيئي الغير حيوية والذي يؤثر سلباً على إنتاج القمح (*Triticum aestivum* L.). تلعب العديد من عائلات عوامل النسخ (TF)، مثل WRKY و MYB و NAC، دوراً مهماً في الاستجابة لإجهاد الجفاف في النباتات. يعتبر البحث عن جينات NaC و WRKY مجالاً جديداً للبحث خاصة في مصر. وبالتالي، هدفت هذه الدراسة إلى اكتشاف تواجد TaNAC2a و TaWRKY2 و TaWRKY19 في تراكيب وراثية مختلفة من القمح، وتحديد ال polymorphism في تتابع منطقة بوسط اكسون ٢ للجين TaNAC2a بين بعض التراكيب الوراثية للقمح المصري. ال DNA marker المعتمد على المنطقة المشفرة استخدم للكشف عن هذه الجينات الثلاث، تم تحليل تتابع المنطقة المركزية من إكسون ٢ في سبعة أصناف من القمح. تم اكتشاف TaWRKY2 و TaWRKY19 و TaNAC2a